REMARKS/ARGUMENTS

Reconsideration of this application and entry of the foregoing amendments are respectfully requested.

The claims have been revised to define the invention with additional clarity and to address points raised by the Examiner in paragraphs 4 and 6-8 of the Action (it will be noted that claims 21 is now presented in independent form). Applicants submit that the revisions of claims 39 and 48 are entirely consistent with the disclosure (particularly, pages 18 and 24). New claims 50-67 are fully supported by an enabling disclosure (new claims 50-63 correspond to claims 16-29 but depend from claim 41; new claims 64 and 65 find support, for example, at pages 18 and 24). Claims 35 and 44 have been cancelled without prejudice.

Claims 16-29, 32, 33, 35, 37-42, 44 and 46-49 stand rejected under 35 USC 112, first paragraph as allegedly being non-enabled. Withdrawal of the rejection is requested.

At the outset, Applicants point out that in the Office Action (final rejection) dated March 25, 2003, the Examiner acknowledged (page 5, last five lines) that:

> [the specification is] "...**enabling** for...CH2 domain...at least 98 [%] identical to a CH2 sequence (residues 231-[3]40) from human IgG1 or IgG2 having said modified amino acids...".

(Emphasis added.)

This language appears in the present claims. However, the Examiner does not appear to repeat this acknowledgement in the present Action. Clarification is requested.

The comments that follow are responsive to specific portions of the Action, as indicated:

i) Page 4 "...only 2 binding molecules..." to Page 5 "...other than..."

The Examiner is respectfully requested to clarify the comments set forth in the paragraph bridging pages 4 and 5 of the Action (page 4 "...only 2 binding molecules..." to page 5 "...other than..."). The specification does not "only" disclose 2 binding molecules. The section on "further discloses...IgG1..." is also seemingly incomplete, i.e., it does not appear to refer to all of the worked examples e.g., in Table 1. Applicants offer the following comments by way of response.

First, as indicated in the Amendment filed in response to the previously issued final rejection (FR), in terms **of disclosure,** the specification discloses several binding domains, e.g., page 15, line 1 – page 24, line 17 – see also Example 7. Without a reason to doubt the truth of the statements made in the patent application, the application must be considered enabling. *In re Wright*, 999 F.2d 1557, 1562, 27 USPQ2d 1510, 1513 (Fed. Cir. 1993); *In re Marzocchi*, 439 F.2d 220, 223, 169 USPQ 367, 369 (CCPA 1971). The burden placed on the Examiner is reflected in the MPEP Section 706.03.

Second, as also indicated in the Amendment filed in response to the FR, even in terms of **worked examples,** mutations in the 233-236 & 327, 330-1 region were prepared in **8 different combinations, which were compared with each other and 3 different wild-type immunoglobulins** in many different experiments (see, e.g., Figures 1 to 14) including more than one variable region for increased confidence. The consistent results were that immunoglobulins including the claimed combinations of residues gave the required properties in both IgG1, 2 and 4 backgrounds and irrespective of which binding domain was used.

Although the Examiner (pages 8-9) acknowledges that these arguments have been made, the Examiner has not specifically addressed these points.

Applicants are deeply concerned that the full extent of the disclosure of the invention, and its commensurate relationship with the claims, has not been acknowledged by the Examiner. Therefore, a point by point comment on the Examples (page 33 onwards) of the present application is set out below:

<u>Construction of expression vectors</u> (General Materials and Methods, pp33-39 and Example 1, p45)

The specification describes a method by which nucleic acids of the invention can be constructed. Human IgG constant region genes or cDNAs and vectors suitable for the expression of antibody genes are available from many sources and several methods for site-directed mutagenesis are commonly known so this section is by way of example only. However, the Examiner's attention is drawn to the detail of the mutations of the invention (pp 35-36), since the manner which they are presented at pages 4 to 5 of the Action is, respectfully, unnecessarily complicated.

The binding molecules of the invention contain **IgG4 residues 327G, 330S and 331S** in their human IgG CH2 domain (as encoded by the oligonucleotides MO22BACK and MO22, for example). Thus, where the parent CH2 domain is the human IgG1 CH2, mutated residues are present at positions 327, 330 and 331 (e.g., G1Δab, G1Δac). Where the parent CH2 domain is the human IgG2 CH2, mutated residues are present at positions 330 and 331, since native IgG2 itself contains 327G (e.g. G2Δa). Where the parent CH2 domain is the human IgG4 CH2, none of the residues 327, 330 and 331 are mutated (e.g. G4Δb, G4Δc).

The binding molecules of the invention **also** contain **IgG2 residues 233P, 234V and 235A** in their human IgG CH2 domain (as encoded by the oligonucleotide MO7BACK, for example). In addition, residue 236 can be deleted as seen in IgG2. This combination of 233P, 234V, 235A and no residue at position 236 is encoded by an oligonucleotide such as MO21. Thus, where the parent CH2 domain is the human IgG1 CH2, mutated residues are present at positions 233, 234 and 235, with the native IgG1 residue 236G (e.g. G1Δac) or with residue 236 deleted (e.g. G1Δab). Where the parent CH2 domain is the human IgG2 CH2, none of the residues 233, 234 and 235 are mutated (e.g. G2Δa). Where the parent CH2 domain is the human IgG4 CH2, mutated residues are present at positions 233, 234 and 235, with the native IgG4 residue 236G (e.g. G4Δc) or with residue 236 deleted (e.g. G4Δb).

DNA encoding antibody variable regions with a desired specificity can be obtained by several methods including reverse transcription and PCR from many sources including antibody-secreting cell lines or the B-cells of an individual. Alternatively, for many desired specificities, antibodies comprising suitable variable regions have been described in the literature and the variable region DNA has already been isolated. This was the case for the Fog-1 variable region DNAs of Example 1. As a further alternative, the variable region DNAs obtained by one of these methods can have then been modified to make the encoded variable domains more suitable for use in humans. This was the case for the CAMPATH-1 variable region DNAs of Example 1, which encode humanized forms of variable regions from a rat antibody. Thus, all methods for obtaining or generating variable regions DNAs in order to take advantage of this invention are not contained explicitly in Example 1 but are well known in the art. Details of the manipulation of the Fog-1 and CAMPATH-1 variable regions are provided merely to allow complete description of sample vectors and are not intended to limit the scope of the invention.

Production of antibodies (General Materials and Methods, pp39-40 and Example 1, p45-47)

Methods for obtaining purified antibody product from cells transfected with the constructed vectors are described. Suitable methods vary according to the design of the vectors, the cell line employed, the scale of antibody production and other factors but are again well known in the art. However, the Examiner's attention is drawn to the care taken in checking the integrity and concentration of the antibodies since this ensures that subsequent measurements of effector functions are meaningful.

The following is an explanation of the results described in Examples 2-6b as illustrated by Figures 1-14. The methods are detailed in General Materials and Methods, pp40-45.

FcγRI binding (Example 2)

The rosetting of FcγRI transfectants is an assay that looks at the ability of the IgG Fc region to interact with the receptor FcγRI. RhD-positive red blood cells are incubated with Fog-1 antibodies which bind to the RhD antigen on the red cell surface via the antigen-binding sites in their variable regions. After washing, the red blood cells are added to FcγRI-expressing cells that have been grown in wells and are adhering to the plastic surface. After incubation, excess red cells are washed away to enable visualization of any rosetting. This can be understood by referring to the schematic cartoon in the **attached Figure I**. In part a, the black Fog-1 antibody contains CH2 domains that are capable of binding to FcγRI. Interactions between the Fc-regions of the antibodies coating the red cells and the receptors on the surfaces of the adhered cells cause the red cells to cluster around the adhered cells, giving a so-called rosette formation. In reality, larger numbers of cells would be involved and the number of IgG bound to the receptor at the contact surface would be much greater. The percentage of FcγRI-bearing cells associated with

red cells is recorded as measure of the strength of the interaction between the coated red cells and the receptor-bearing cells. Thus, as seen in **Figure 1** of the application, the interaction between the CH2 domains of the G1, G1Δa and G4 antibodies and FcγRI is sufficient to enable the majority of FcγRI-bearing cells to form rosettes when the red cells were coated with concentrations of antibody above 1 μg/ml. Since rosette formation is a function of both the strength and number of interactions, the percentage of FcγRI cells able to form rosettes falls as the antibody concentration decreases. In part b, the white Fog-1 antibody contains CH2 domains that are incapable of binding to FcγRI. Even when the red cells are coated with a high density of antibody there is no rosette formation. This was the case when testing the IgG2 wild type antibody and all mutants except for G1Δa. Thus this assay was unable to detect any interaction between FcγRI and the Fc regions relating to G1Δab, G1Δac, G2Δa, G4Δb and G4Δc of this invention.

FcγRI is a high affinity receptor for IgG and the binding of monomeric IgG molecules can be detected. While the rosetting assay described above is a measure of the strength of interaction between an Fc and FcγRI, it can only be used in this format when the Fc can be attached to red blood cells. A further assay detects antibodies bound to FcγRI molecules on receptor-bearing cells using a fluorescent reagent and measurement of the level of fluorescence of the cells. The cartoon of the **attached Figure II** demonstrates the principle of the assay, focusing on a token number of FcγRI on one cell of the population. In the first incubation, test antibodies of any antigen-binding specificity which are able to bind to FcγRI become held by the receptor (part a) and remain associated with the cell in subsequent washing steps. Antibodies not able to bind to FcγRI remain free in solution (part b) and are then washed away. In the second incubation, the biotin-labeled detecting antibody recognizes the light chain of the bound test

antibody (part a) and remains on the cell after washing. When there is no test antibody on the cell (part b), all of the detecting antibodies are washed away. In the final incubation, ExtrAvidin-FITC binds to the biotin label present in part a but can all be washed away in part b. The number of FITC molecules attached to the cell determines the level of fluorescence that can be measured.

Figure 3 of the application shows fluorescence histograms for cells incubated with selected CAMPATH-1 antibodies at 100 μg/ml, the binding of which was then detected as described. For each fluorescence level, the number of cells (events) having that level of fluorescence is plotted. For each antibody a peak is obtained on the histogram, the variation around the mean of this peak due to the slight differences in the amount of FcγRI expressed by individual cells in the population. The further to the right the peak, the higher the average level of fluorescence and thus the higher the level of FcγRI binding by the test antibody. High levels of G1, G1Δa and G4 were detected binding to the cells whereas cells incubated with G1Δb show low fluorescence, equivalent to the natural fluorescence of the cells and indicating that no antibody bound to the receptor.

Figure 2 of the application shows the results of such assays using sets of antibodies with either CAMPATH-1 (a and c) or Fog-1 (b and d) variable regions, each on two types of cells transfected to express FcγRI. Here the mean fluorescence activities are plotted against the test antibody concentration so that the amount of active antibody (G1, G1Δa or G4) binding is seen to fall as its concentration is reduced. Of the other antibodies, only very low levels of binding are seen above background and only at high antibody concentrations. Thus, these results support the conclusions of the rosetting assay of **Figure 1** of the application.

This fluorescence staining assay purely measures binding to FcγRI and is independent of the specificity of the variable regions of the antibody. In Appendix I (data first provided in the Amendment filed January 2, 2003), the behavior of three constant regions of the invention (G1Δab, G1Δac and G2Δa) in this assay is shown to be independent of variable region binding specificity; the mutations having the same effect on binding to FcγRI in the context of different variable regions. This includes the antibodies with specificity for human platelet antigen-1a (HPA-1a; see Example 7), which use a lambda light chain and the binding of which was thus detected using an anti-human lambda light chain detection reagent.

<u>FcγRII binding</u> (Example 6)

In contrast to FcγRI, the lower affinity of receptors of the FcγRII and FcγRIII classes means that binding of monomeric IgG cannot be detected. To increase the avidity of the interaction so that a fluorescence staining method could be used, $F(ab')_2$ fragments of goat antibodies recognizing the light chain of the test antibodies were used to cross-link the test antibodies into complexes. This is demonstrated schematically in the **attached Figure III**. This allows each complex to present multiple Fc regions to the receptors. If the Fc regions are able to bind to the receptor molecules (part a), complexes become attached to the cells and are not washed away. If the Fc exhibits a lower affinity for the receptor such that even complexes of the antibodies cannot be captured by the cells then the complexes are removed on washing (part b). Detection of any bound complexes is achieved via a FITC-labeled antibody which recognizes the goat cross-linking reagent (as shown in the final incubation step of **Figure III**) or by using a directly labeled cross-linking reagent.

Assays measuring FcγRII binding are illustrated in **Figures 13a, 13b and 14a** and more examples, together with a detailed discussion, have been published (Armour, K.L., van de

Winkel, J.G.J., Williamson, L.M., & Clark, M.R. (2003) Differential binding to human Fc gamma RIIa and Fc gamma RIIb receptors by human IgG wildtype and mutant antibodies. *Molecular Immunology*, 40:585-593.). While the Δb and Δc mutations in particular reduce binding to FcγRII, the reduction in binding relative to the parent constant region is greater for the two allotypic forms of FcγRIIa (131H and 131R) than for the inhibitory receptor FcγRIIb. This can be seen by reference to Figure 16 and in Appendix I where the relative reductions in binding given are based on measurements on the context of three different antigen-binding specificities.

FcγRIII binding (Example 6b)

Receptors of the FcγRIII class are also low affinity and binding of antibody complexes to FcγRIIIb receptors of the NA1 and NA2 allotypes was measured using the method illustrated in **Figure III**. None of the molecules of the invention (G1Δab, G1Δac, G2Δa, G4Δb and G4Δc) show binding above background to either receptor. This result is reiterated in Appendix I for G1Δab, G1Δac and G2Δa each with two different antigen-binding specificities.

Monocyte activation (Example 3)

The activation of monocytes in response to cells coated with antibody is mediated through FcγRI and FcγRIIa. The level of activation can therefore reveal functional consequences of reducing binding to these two receptors. A cartoon demonstrating the principle of the assay can be found in **Figure IV**, part a. Target cells (RBC) are pre-sensitized with antibodies, which bind to the cell through their antigen-binding sites. The sensitized cells are then mixed with monocytes in the presence of luminol. When the antibody Fc regions have sufficiently strong interactions with FcγRI and/or FcγRIIa and the Fc are presented to the monocyte at a high enough density, binding of the Fc regions to their receptor causes clustering of the FcγR and

activation of the monocyte. A by-product of the activation and phagocytosis processes reacts with luminol to give chemiluminescence (CL), which can be measured. The CL mediated by the active antibodies G1, G1Δa and G4 is quantified in **Figure 4**. When these antibodies are presented at lower densities on the red cell surface, less clustering of the FcγR occurs and the CL level is lower. For the antibodies G1Δb, G1Δab, G2, G2Δa, and G4Δb, any interactions between the Fc and FcγR are too weak to maintain the red cells and monocytes in close contact even at the highest densities of antibody on the red cell surface (depicted in **Figure IV**, part b). Since no clustering of FcγR occurs, the monocytes are not activated and there is no CL signal. Antibodies G1Δc, G1Δac and G4Δc show some monocyte CL but, at the highest densities of coating, this only amounted to the level of CL seen for the lowest amount of the active antibodies such as G1. As Appendix I indicates, data are now available concerning the level of monocyte CL in response to platelets sensitized with anti-HPA-1a antibodies. Anti-HPA-1a versions of the G1Δab and G1Δac antibodies also mediated vastly reduced CL in comparison to anti-HPA-1a G1. The low levels of CL observed with high amounts of the variant antibodies could be achieved with 100-fold less of the G1 antibody.

The ability of the molecules of the invention to block CL evoked by active antibodies has also been measured since this mimics a proposed use of the molecules as *in vivo* blocking antibodies. Part c of **Figure IV** illustrates the principle. The target cells (e.g., RBC or platelets) are sensitized with a mixture of active and inactive antibodies. Occupancy of antigen sites by the inactive antibody will decrease the amount of active antibody which would otherwise bind, with the relative densities of the antibodies on the cells depending on the quantities of each used. Thus the target cell surfaces seen by the monocytes have a lower density of Fc regions which

can engage the FcγR. **Figure 5** shows how the variant antibodies are able to reduce the CL due to G1 when the Fog-1 antibodies are used to sensitize RBC. In fact, although G2 antibody is inactive in CL (**Figure 4**), the molecules of the invention are more effective at inhibiting G1-mediated CL. It is thought that when G1 antibody is present and can promote cell-cell association via its interaction with the high affinity FcγRI, G2 is able to interact with FcγRIIa sufficiently to maintain the CL signal until G1 levels are further reduced. Thus this assay demonstrates a functional consequence of the reduced FcγRIIa binding shown by molecules of the invention.

One of the variant Ab, Fog-1 G2Δa, was also shown to inhibit the CL response to clinically significant anti-RhD Ab in human sera (**Figure 6**). The CL assay has been developed as a means of using maternal serum to predict the severity of haemolytic disease of the fetus. Results above 30% on this scale are associated with hemolysis and suggest invasive antenatal intervention procedures are appropriate. Inclusion of the G2Δa Ab effected a reduction in the CL response to below this 30% threshold for each anti-RhD serum, but not for sera containing antibodies to other RBC antigens, demonstrating the specificity of the blocking. These results, measuring the ability of the molecules of the invention to block CL evoked by active antibodies, have been confirmed for the anti-HPA-1a G1Δab and G1Δac antibodies using sensitized platelets.

<u>Complement-mediated lysis</u> (Example 4)

Like monocyte activation, complement mediated lysis is also dependant on the density of antibody on the cell surface. In the case of the classical pathway of complement activation, C1q molecules need to be able to bind to multiple Fc regions to initiate the complement cascade.

Since anti-RhD antibodies bound to RBC never activate complement, the CAMPATH-1 series of antibodies were tested for their ability to mediate lysis of peripheral blood mononuclear cells (PBMC) in the presence of autologous serum as a source of complement. As shown in **Figure 7,** G1 and G2 cause lysis in a concentration-dependant manner. None of the molecules of the invention mediate lysis above background at any of the antibody concentration tested. This result has been confirmed for an anti-VAP-1 G2Δa antibody (Appendix I and **Figure V**).

As another way of mimicking the use of the molecules as *in vivo* blocking antibodies, the ability of CAMPATH-1 G2Δa to block complement lysis mediated by CAMPATH-1 G1 was also tested (**Figure 8**). As in the CL assays, the presence of the inactive antibody, competing for antigen sites, reduces the density of active Fc on the target cell surface. Thus, increasing concentrations of G2Δa are able to reduce the lysis to background levels.

<u>Antibody-dependant cell mediated cytotoxicity</u> (Example 5)

ADCC was measured using PBMC target cells for the CAMPATH-1 antibodies (**Figure 9**) and RhD-positive RBC for the Fog-1 antibodies (**Figures 10a and 10b**). ADCC is thought to be mediated through FcγRIIIa on NK cells. Levels of ADCC mediated by a particular antibody can vary considerably between effector cells from different donors, as demonstrated for the G2 and G4 wildtype antibodies in the experiments of **Figures 10a and 10b**. However, the molecules of the invention (G1Δab, G1Δac, G2Δa, G4Δb and G4Δc) were unable to mediate hemolysis with effector cells from any of the donors used.

In vivo use of blocking antibodies was again mimicked by using variant antibodies to inhibit ADCC mediated by active antibodies. As previously, the antibodies will compete for RhD sites on the red cells so that the density of the active antibody is reduced. **Figure 11a and**

11b show reduction in Fog-1 G1-mediated ADCC with Fog-1 G1Δab, G1Δac, G4Δb and G4Δc being among the antibodies that most efficiently inhibited lysis. **Figure 12** shows that G1Δab and G4Δb also efficiently blocked the lytic activity of the antibodies in a clinically-relevant anti-RhD serum.

ii*) Page 5 "...Other than..."*

Applicants submit that once again in asserting that the teaching is limited to the precise examples (indeed less than the precise examples seemingly) the Examiner is ignoring the fact that the specification includes general teaching and is addressed to one skilled in the art.

In section (i) above Applicants have discussed the disclosure of the Examples. However, the Examiner's attention is directed to the fact that further examples of molecules of the present invention have also been provided and tested, and their properties are consistent with the teaching of the invention.

In the Amendment filed January 2, 2003, data on further variable regions was provided. This is discussed above, and reproduced again below for ease of reference as **Appendix I.** Additionally, attached **Appendices II – IV** give further illustrations of the functional utility of molecules of the invention.

In summary, Applicants have not only shown that the molecules of the invention have decreased binding to activating FcγR and decreased abilities in mediating killing mechanisms but have also shown the molecules of the invention to be efficient at inhibiting destructive functions of active antibodies. For selected molecules of the invention, they have made antibodies with three different variable region specificities and have shown the characteristics of the constant

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region to be independent of the variable region. They have shown binding to FcRn to be unaffected by the mutations. The evidence consistently shows that the teaching of the invention (i.e., optimized FcγR-binding achieved by use of molecules having constant regions with the specified novel combinations of amino acids) is **generic in nature** (i.e., "fit for generalization") and does **not** simply pertain to the specific assays and molecules described in the Examples section of the specification.

As discussed above, given the teaching in the application, the skilled person can prepare the modified antibodies having the specified sequences. It is also apparent that having prepared these antibodies, the skilled person can formulate these as pharmaceuticals and use them analogously to the well established use of therapeutic antibodies in the prior art.

iii) Page 5 "...the following reasons."

"First...binding specificity"

The claims require that the "binding molecule" have "a binding domain...which is the binding site of an antibody". However they do not even recite the term "binding specificity". In any case, as the Examiner is aware, the present invention is not about novel binding specificities (i.e., the variable region of antibodies) but rather is about novel effector domains (i.e., the constant region of antibodies). The effector domains of the invention can be freely combined with conventional binding domains for use in the methods of the invention.

The Examiner contends that there is "insufficient guidance" as to the "binding specificity" and the target, but does not say why she believes this is necessary to practice the invention, nor why she believes it is insufficient. As discussed above, and supported by the

evidence in the application and provided subsequently, the invention is generic in nature (not limited to particular specificities) and the claims are worded accordingly.

The Examiner has not provided the required explanation/evidence to support her assertion that the skilled person could not make and use the invention as claimed.

iv) "Second...structure of the..."

In terms of structure, claim 32 (for example) requires that the effector domain be "a chimeric domain which is derived from two or more human immunoglobulin heavy chain C_H2 domains, which human immunoglobulins are selected from IgG1, IgG2 and IgG4...and [which] is a human immunoglobulin heavy chain C_H2 domain which has the following blocks of amino acids at the stated positions: 233P, 234V, 235A, 236G, 327G, 330S and 331S numbered with respect to the EU numbering system of Kabat, and is at least 98% identical to a C_H2 sequence (residues 231-340) from human IgG1 or IgG4 having said modified amino acids"

In making the rejection, it appears the Examiner has simply paraphrased the claim and asserted that there is "insufficient guidance" about the "structure" of the remaining features, e.g., ignoring that the chimeric domain and human C_H2 domains are clearly defined as above.

Again, the Examiner is urged to provide the required evidence or reasoning to support the assertion that the skilled person could not make and use the invention as claimed.

v) "Third\Fourth...combination of.... [claim 35]

Purely for procedural expediency, claim 35 has been cancelled.

vi) Page 6 "Since the binding specificity of the ..." [claims 16, 17]

Applicants submit that as the binding molecule and effector domains are enabled, the nucleic acids are also enabled.

vii) "Fifth...modified nucleic acids....

Purely for procedural expediency, claims 16 and 17 have been revised to indicate that the nucleic acid is DNA.

viii) "In addition to the lack of guidance...which nucleotides"

The Examiner's comment is not understood and Applicants respectfully request that the Examiner clarify her concern so that they can properly respond. However, inasmuch as it is about the Kabat numbering, this is the accepted manner of numbering constant regions and there can be absolutely no doubt that the amino acids 233-236 & 327-331 can be assigned using this system.

Claim 21 was dependent on claim 32 so the identity of the amino acids in this region are fixed. Nevertheless, in order to progress prosecution, claim 21 has been written in independent form in order to clarify which codons are modified.

ix) "Further, there is insufficient guidance as to which amino acids...."

While Applicants are unclear as to the nature of the Examiner's concern, it may be rendered moot by the above-noted amendment of claim 21.

x*) Page 6, last line "Given the..." to page 7 first paragraph*

The Examiner objects to there being insufficient working examples. The Examiner's attention is directed to the comments offered above in (i). It will be clear from those comments that there are sufficient working Examples to justify the scope of the claim.

xi) Page 7 "Further there is a lack of in vivo working examples..."

The comments that follow correspond to those previously presented but not addressed by the Examiner. The Examiner is urged to give careful consideration to Applicants remarks and provide an explanation if she finds them to be insufficient.

The presently claimed molecules have been tested for numerous effector functions as described in Figures 1 to 14 and Examples 1 to 6b. Furthermore, they have been shown to inhibit the response of monocytes to immunoglobulins sensitized cells and inhibit the killing of targeted cells through complement lysis or ADCC. As discussed in the Examples, e.g., page 49, lines 30-34, the tests used have been those already shown to be useful in predicting *in vivo* pathology. The tests on page 50 studied CL responses which were indicative of hemolytic disease in the newborn.

Therefore, *prima facie* both generally and specifically it is reasonable to assume the *in vivo* utility of the presently claimed immunoglobulins. The 3 "reasons" given on page 12, lines 5-9 of the FR are entirely speculative and completely unsupported by any of the **evidence**, particularly in view of the widely recognized and well established use of antibodies for therapy.

The Examiner's objections are simply that the claims are broader than the examples, but that alone is not a ground of rejection under 35 USC 112, first paragraph. Further, working *in vivo* examples are **not** required by the statute, rules, or the case law. Indeed as stated in *Cross v. Iizuka*, 753 F.2d 1040, 1050, 224 USPQ 739, 747 (Fed. Cir. 1985), all that is required is that:

> "...based upon the relevant evidence as a whole, there is a reasonable correlation between the disclosed *in vitro* utility and an *in vivo* activity, and therefore a rigorous correlation is not necessary where the disclosure of pharmacological activity is reasonable based upon the probative evidence."

Although the Examiner (at pages 8-9) acknowledges that Applicants have advanced these arguments, the Examiner has not **specifically addressed** Applicants' points.

In addition, the Examiner's attention is directed to **Appendix III** below which discusses a human volunteer study.

xii) "Even if the binding molecule..."

This aspect of the rejection is unclear. As Applicants understand it, the Examiner is asserting that even the sequences for which there are worked examples are not enabled because the specification states:

"the effect of mutations cannot always be predicted from wildtype antibody activities..."

In response, Applicants offer the following.

First, it is not clear to what mutations the Examiner is referring. SEQ ID NOs 1-3 (for example) are, of course, fully defined. Clarification is requested.

Second, even considering the scope of the claims, there is no question of predicting the (effector) activity from <u>wild-type</u> antibodies. As noted in (i), Applicants have provided numerous examples of mutants (SEQ IDs 1-3, and 8-11), both inside and outside of the scope of the claims. The <u>mutants</u> present a consistent picture (albeit one that could not have been 'predicted' from the prior art) of the effector functions achieved by the combination of V,A,G...G,S,S recited in the claims.

With respect to the comments about G1Δb & G1Δc and page 55, it should be noted that these do not fall within the present claims – see Figure 15, Table 1 (these molecules have A,A,P at 327,330,331). Therefore, the Examiner's point in referring to page 55 is not understood and clarification is requested.

In regards Mogan (*sic*), Ngo, Kuby, Riechmann, Applicants have <u>previously pointed out</u> that the fact pattern of the present case does not match the fact pattern of these disclosures:

> "The disclosure of Morgan *et al.* cited by the examiner on page 9, lines 26-28 likewise refers to position 235 which is 'fixed' in the claims. Of course one could equally point to, say, Morgan's K320A which does not appear to affect the activities of IgG1 (see Table 2) and deduce that changes outside of 233-236 & 327, 330-1 do <u>not</u> affect properties...
>
> ...In respect of Ngo *et al.*, we point out for completeness that we are not attempting to define structure purely by reference to activities (cf. TRAINING MATERIALS FOR EXAMINING PATENT APPLICATIONS WITH RESPECT TO 35 U.S.C. SECTION 112, FIRST PARAGRAPH-ENABLEMENT - CHEMICAL/BIOTECHNICAL APPLICATIONS, Example N: DNA). In any case, by contrast with Ngo *et al.,* the crystal structure of IgG is known (e.g. Clark, 1997 of record – page 92 and legend page 93), and there is a considerable literature on it. Certainly in the light of this and the present disclosure it would be possible for the skilled person to predict residues which are non-sacrosanct in the claims, and which would not be expected to destroy activities e.g. because they

> were not near the sacrosanct residues, or because they were conservative substitutions.
>
> The reference to Kuby *et al.*, seems irrelevant since it does not seem to be concerned with engineering immunoglobulins."

Considering Riechmann, the passage pointed out by the Examiner merely states that the wild-type IgG antibodies have different effector functions. With respect, this has no bearing on whether the present claims (limited to a particular class of mutants) are enabled.

xiii) "Applicant's arguments filed 1/26/04..." to page 11

The Examiner appears to reiterate Applicants' response without comment.

xiv) Page 11 "However, the scope of the claims..."

The Examiner has not specifically addressed Applicants' arguments, but rather just notes that the claim is generic, i.e., relates to a class of binding molecules. Applicants acknowledge that the claim is generic but the Applicants' arguments addressed precisely why the generic claim should be allowable.

As to "...treating a patient with all disorders such as the ones..." Applicants do not claim that the binding molecules are some wonder-drug capable of treating all disorders. Respectfully, the Examiner is apparently mis-construing the invention.

xv) Page 11 "The specification discloses only..." to Page 15, pt. 11

This merely repeats points addressed in (i) to (xii) above. Applicants again submit, the Examiner has not specifically responded to the arguments made previously.

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In view of the above, the Examiner is urged to reconsider the rejection and, in so doing, Applicants request that the Examiner give careful consideration to the full extent of the disclosure provided. It is believed that, having done so, the Examiner will find the claims to be fully supported by an enabling disclosure. Should the Examiner be inclined to maintain the rejection, she is requested to point out with specificity what it is she finds lacking in the responsive comments provided.

Claims 16-29, 32, 33, 35, 37-42, 44 and 46-49 stand rejected under 35 USC 112, first paragraph, as allegedly lacking written description. Withdrawal of the rejection is submitted to be in order for the reasons that follow.

Applicants offer the following remarks responsive to specific comments provided by the Examiner in support of the rejection.

xvi) Page 15 "The specification does not reasonably provide a written description..."

This is mere assertion without support. Nevertheless, claim 21 has been amended as discussed above.

xvii) Page 16 "The specification discloses only 2 binding molecules..."

The comments offered in (i) above are applicable here and the Examiner is requested to consider same.

xviii) "With the exception of..."

Examiner asserts there is "inadequate written description" about binding specificity and so forth. As noted in (iii) above, the claims do not even use the term "binding specificity" – note, however comments in (xix) below.

As regards the remaining points raised by the Examiner on pages 16 and 17 of the Action, attention is directed to the comments offered in (vi) – (ix) above.

xix) Page 18, "in response to"

Examiner has reiterated the comment about "binding specificity", apparently in response to Applicants' reference to Example 16 of the "Synopsis". The synopsis was cited as follows:

> "The general knowledge in the art is such that antibodies are structurally well characterized. It is well known that all mammals produce antibodies and they exist in five isotypes, IgM, IgG, IgD, IgA and IgE. Antibodies contain an effector portion which is the constant region and a variable region that contains the antigen binding sites in the form of complementarity determining regions and the framework regions. The sequences of constant regions as well as the variable regions subgroups (framework regions) from a variety of species are known and published in the art. It is also well known that antibodies can be made against virtually any protein."
>
> The claim in question was: An isolated antibody capable of binding to antigen X."

The point Applicants wish to make is that the claim in the teaching materials is not limited to particular constant regions, but rather defined by its contribution to the art – in that case a spectrum of novel antibodies characterized purely by their variable regions. The example is thus deemed to satisfy the written description requirement, presumably because the sequences of constant regions were known and published. Therefore, there was no requirement for the claim to be limited to particular examples.

The present case represents a the parallel situation. Applicants submit that there is no justification for requiring that the claims be limited to particular variable regions for precisely the reasons given in the Synopsis, that is:

> "the sequences of… variable regions subgroups (framework regions) from a variety of species are known and published in the art. It is also well known that antibodies can be made against virtually any protein."

xx) "The scope of the claims…"

This paraphrasing of the claims appears to omit some of the functional limitations.

xxi) "The specification does not reasonably provide…"

This is mere unsupported assertion.

xxii) "The specification discloses only…"

The Examiner's attention is directed to the comments offered in (i) above.

xxiii) Page 19. "With the exception of the..."

The Examiner's attention is directed to the comments offered in (xxviii) above..

*****

Reconsideration and withdrawal of the of the rejection are requested.

Claims 16-29, 32, 33, 35, 37-42, 44 and 46-49 stand rejected under 35 USC 112, second paragraph, as allegedly being indefinite. Withdrawal of the rejection is submitted to be in order for the reasons that follow.

xxiv) Page 21 "The "...binding molecule...""

Claims 32 and 41 have been revised to clarify that it is the effector domain that binds the receptors.

xxv) "The "numbered with respect to...""

With respect, Applicants submit that the EU system of Kabat (Kabat EA, Wu TT, Perry HM, et al. Sequences of proteins of immunological interest. USDepartment of Health and Human Services, U.S. Government Printing Office, 1991) provides a **standard** numbering system, and, therefore, an actual wild-type sequence does not need to be recited.

xxvi) "The "homologous..."

Claims 32 and 41 have been revised to remove the "all or part" feature.

xxvii) "The "G1Δab or G2Δa..."

SEQ IDs have been added to claim 42.

xxviii) "The "effector domains ..."

Claims 33 and 42 have been revised to reflect the language of claims 32 and 41.

****

In view of the above, reconsideration is requested.

This application is submitted to be in condition for allowance and a Notice to that effect is requested.

Respectfully submitted,

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Appendix I – data provided in applicant's response filed January 2, 2003

Effects of Δab and Δac mutations on IgG1 activity and of Δa mutation on IgG2 activity in various test systems. The variable regions contexts in which the constant regions were tested are listed. They are CAMPATH-1 (CD52 specificity), Fog-1 (anti-RhD), B2 (anti-human platelet antigen-1a) and 2D10 (anti-vascular adhesion protein-1). The reductions in activity given represent the approximate increase in antibody concentration necessary to achieve the same level of activity as IgG1 or IgG2.

IgG1Δab = 233P, 234V, 235A and 236- and 327G, 330S and 331S present in the tested molecule.

IgG1Δac = 233P, 234V, 235A and 236G and 327G, 330S and 331S present in the tested molecule.

IgG2Δa = 233P, 234V, 235A and 236- and 327G, 330S and 331S present in the tested molecule.

(see patent application Figure 15)

Function	Effect on Δab mutation on IgG1 activity	Variable regions with which tested
Binding to:		
FcγRI	$\geq 10^4$-fold reduction to background	CAMPATH-1, Fog-1, B2
FcγRIIa 131R	8-fold reduction	CAMPATH-1, Fog-1, B2
FcγRIIa 131H	16-fold reduction	CAMPATH-1, Fog-1, B2
FcγRIIb1*	4-fold reduction	CAMPATH-1, Fog-1, B2
FcγRIIIb NA1	>100-fold reduction to background	Fog-1, B2

FcγRIIIb NA2	>100-fold reduction to background	Fog-1, B2
Monocyte activation	≥100-fold reduction	Fog-1, B2
Complement lysis	>50-fold reduction to background	CAMPATH-1
ADCC	>100-fold reduction to background	CAMPATH-1, Fog-1

Function	Effect on Δac mutation on IgG1 activity	Variable regions with which tested
Binding to:		
FcγRI	≥10^4-fold reduction to background	CAMPATH-1, Fog-1, B2
FcγRIIa 131R	10-fold reduction	CAMPATH-1, Fog-1, B2
FcγRIIa 131H	32-fold reduction	CAMPATH-1, Fog-1, B2
FcγRIIb1*	4-fold reduction	CAMPATH-1, Fog-1, B2
FcγRIIIb NA1	>100-fold reduction to background	Fog-1, B2
FcγRIIIb NA2	>100-fold reduction to background	Fog-1, B2
Monocyte activation	≥100-fold reduction	Fog-1, B2
Complement lysis	>50-fold reduction to background	CAMPATH-1
ADCC	>100-fold reduction to background	CAMPATH-1, Fog-1

Function	Effect on Δa mutation on IgG2 activity	Variable regions with which tested
Binding to:		
FcγRI	remained at background	CAMPATH-1, Fog-1, 2D10
FcγRIIa 131R	no effect	CAMPATH-1, Fog-1, 2D10
FcγRIIa 131H	8-fold reduction	CAMPATH-1, Fog-1, 2D10
FcγRIIb1*	no effect	CAMPATH-1, Fog-1, 2D10
FcγRIIIb NA1	remained at background	Fog-1, 2D10
FcγRIIIb NA2	remained at background	Fog-1, 2D10
Monocyte activation	remained at background	Fog-1
Complement lysis	>20-fold reduction to background	CAMPATH-1, 2D10
ADCC	100-fold reduction	CAMPATH-1, Fog-1

Appendix II – functional consequences of decreases FcγR binding in molecules of the invention

Further demonstration of the functional consequences of decreases in FcγR binding have been shown in an assay which measures the capture of flowing neutrophils via the binding of stationary phase antibody to Fc receptors on the cell surfaces. Recombinant VAP-1 antigen was used to coat microslides and anti-VAP-1 antibody added at 10 μg/ml to form *in situ* immune complexes. PBS was used in place of antibody as a negative control. Neutrophils were perfused for three minutes at physiological shear stress followed by a one minute perfusion of cell-free buffer. The number of neutrophils forming adhesive interactions with the immune complexes (**Figure VI**, part a) and the number remaining adhered to the surface of the microslide after PBS washout (**Figure VI**, part b) were counted. From both of these measurements, it is seen that anti-VAP-1 G2Δa displays no activity, giving the same values as the negative control. Thus, its activity is much lower than G1 and is reduced in comparison with G2. G2Δa was also more effective then G2 at inhibiting adhesion due to G1 in a blocking version of this assay.

Appendix III – lack of destructive effector activity of molecules of the invention

Results demonstrating the lack of destructive effector activity of molecules of the invention *in vitro* have been reinforced in a human volunteer study. This set out to establish whether the properties resulted in improved intravascular survival of cells.coated with a null allotype version of the Fog-1 G1Δab antibody (G1Δnab)[1]. To obtain comparative red cell survival data for Fog-1 wild type IgG1 and G1Δnab antibodies without the added complexity of inter-donor variation, we utilised a dual labelling technique. Two aliquots of autologous RhD-positive red cells, labelled with two different isotopes (^{51}Cr and ^{99m}Tc), were coated with either Fog-1 G1 or G1Δnab to equal levels. This strategy permits assessment of red cell intravascular survival, measurement of ^{51}Cr-activity associated with the plasma to follow cell destruction and gamma camera imaging to assess sites of destruction of ^{99m}Tc-labelled cells. After reinjection, whole blood sample counting showed complete, irreversible clearance of IgG1-coated RBC by 200 min (half-times 15 – 26 min), concomitant with appearance of ^{51}Cr radiolabel in the plasma (**Figure VII**, part a). Gamma camera imaging of ^{99m}Tc -labelled cells revealed accumulation in the spleen and, at higher coating levels, in the liver. In contrast, G1Δnab-coated cells cleared more slowly and incompletely, with whole blood counts falling to 7 – 38% injected dose by about 200 min before increasing. There was no appearance of ^{51}Cr radiolabel plasma (**Figure VII**, part b) and imaging showed no liver clearance. **Figure VIII** compares the variation in whole blood

1. For the avoidance of doubt, the changes in these "null" antibodies compared to those tested in the application as filed are all in the CH1 and CH3 domains, i.e., not in the CH2 domain which is the subject of the invention. Therefore, the data is unequivocably representative of the invention i.e. the "null" changes will not alter the receptor binding effects of the modifications of the invention.

count with time for the two types of cell in one of the volunteers. These findings suggest that RBC coated with Fog-1 G1Δnab were not destroyed but temporarily sequestered in the spleen.

Appendix IV – FcRn binding of molecules of the invention

Recombinant soluble FcRn was made by synthesising the extracellular domains of the α-chain in *E. coli* and refolding the purified material with β2m (β2microglobin). Plasmon resonance measurement of the binding of the recombinant FcRn to antibody immobilised on chips showed the characteristics expected of FcRn. Binding was Ph-dependant, with binding at pH 6.0 and no binding at pH 7.4, and was specific for IgG. Equal amounts of the anti-HPA-1a antibodies G1, G1Δab and G1Δac (null allotype versions designated G1Δnab and G1Δnac) were immobilised onto separate chips. Binding of FcRn to G1 and G1Δnab (**Figure IX**, part a) and G1 and G1Δnac (Figure IX, part b) was measured at the same time to allow direct comparison. An increase in refractive index indicates that additional material is being bound on the chip surface. Thus, the initial curves show binding of FcRn to immobilised antibody with the spikes at 11-12 min occurring when the buffer was changed for one not containing FcRn. The degree of binding is exceedingly similar for the pair of antibodies in each experiment, with the slight offset in time being due to separate additions to the two cells. Thus, as expected, FcRn binding has not been affected by Δab and Δac mutations of IgG1. This makes it highly unlikely that any of the separate mutations Δa, Δb or Δc would influence FcRn binding.

Further, for Fog-1 G1Δnab (null allotype version of G1Δab), we have measured FcRn binding in a functional assay (**Figure X**). The monolayer transport model used cell lines which express FcRn (JAR, derived from syncytiotrophoblast, and HepG2, from liver) and which are grown on filters inserted in wells. IgG is added to the apical side of the filter and the amount of antibody transported across is measured. A small molecule is included to exclude the possibility of leakage (asp). The transport of G1Δnab and G1 are similar when measured over a range of

time periods. The IgG and IVIG measurements are not directly comparable due the presence of different IgG subclasses. Thus, the Δa and Δb mutations do not affect this function.